Altered retinoid signaling compromises decidualization in human endometriotic stromal cells

Mary Ellen Pavone, Saurabh Malpani, Matthew Dyson and Serdar E Bulun

Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

Correspondence should be addressed to M E Pavone; Email: m-pavone@northwestern.edu

Abstract

Decidualization alters multiple molecular pathways in endometrium to permit successful embryo implantation. We have reported that paracrine factors, including retinoids, secreted from progesterone-treated endometrial stromal cells, act on nearby epithelial cells to induce the estradiol metabolizing enzyme HSD17B2. This same induction is not seen in endometriotic stromal cells. We have also shown significant differences in retinoid uptake, metabolism and action in endometriotic tissue and stromal cells compared to normal endometrium. Here, we characterize retinoid signaling during decidualization in these cells. Endometrial and endometriotic cells were isolated, cultured and incubated and decidualized. Genes involved in retinoid metabolism and trafficking were examined using RT-PCR and Western blotting. Prolactin, a decidualization marker, was also examined. We found that both endometrial and endometriotic stromal cells express all intracellular proteins involved in retinoid uptake and metabolism. Decidualization significantly reduced the expression of the genes responsible for retinoid uptake and shuttling to the nucleus. However, expression of CRBP1, an intracellular carrier protein for retinol, increased, as did RBP4, a carrier protein for retinol in the blood, which can function in a paracrine manner. Secreted RBP4 was detected in the media from decidualized endometrial cells but not from endometriotic cells. We believe that retinoid trafficking in endometrial stromal cells during decidualization may shift to favor paracrine rather than intracrine signaling, which may enhance signaling to the adjacent epithelium. There is blunting of this signaling in endometriotic cells. These alterations in retinoid signaling may help explain the decidualization defects and deficient estradiol inactivation (via HSD17B2) seen in endometriosis.

Reproduction (2017) 154 207–216

Introduction

Endometriosis, an estrogen-dependent disorder, is often associated with pelvic pain and subfertility (Béliard et al. 2004, Giudice & Kao 2004, Kennedy et al. 2005, Wu et al. 2008, Nasu et al. 2009). It is estimated that 10% of all women and up to 30–50% of symptomatic premenopausal women are affected by endometriosis, representing around 176 million women worldwide (Giudice & Kao 2004, Bulun 2009, Adamson et al. 2010, Bulletti et al. 2010, Nnoaham et al. 2011, Stilley et al. 2012). A recent study confirmed a high economic burden on endometriosis patients similar to that of other life-altering conditions such as diabetes and Crohn’s disease (Simoen et al. 2012).

Although endometriosis is a known cause of infertility or subfertility, there is debate as to whether it affects oocyte development, embryogenesis or implantation. Studies investigating the effect of endometriosis on IVF outcomes are useful because they allow for the study of surrogate markers of reproductive success including peak estradiol levels, number of oocytes retrieved, fertilization, implantation as well as pregnancy rates. By evaluating each component, it may be possible to determine the specific effects of endometriosis on reproductive outcomes. Barnhart and coworkers published a meta-analysis examining the effect of endometriosis on IVF outcomes (Barnhart et al. 2002). The authors found that endometriosis negatively affected all aspects of IVF outcomes, including implantation rates. Furthermore, when comparing women with endometriosis to women with tubal factor infertility, all aspects of IVF outcome, including implantation rates, were lower (Barnhart et al. 2002). This study also showed that disease severity also affected these outcomes and that women with severe disease had a decrease in most aspects of IVF outcomes including implantation rates (Barnhart et al. 2002).

Reduced implantation as well as altered receptivity in the endometrium may be caused by reduced capacity to decidualize. Decidualization is the response of maternal cells to progesterone in preparation for arrival of the blastocyst in early stages of pregnancy (Dunn et al. 2003). During this process, endometrial remodeling takes place by a secretory transformation of uterine glands, an influx of specialized uterine natural killer cells, and
vascular remodeling (Gellersen et al. 2007). Klemmt and coworkers found that endometriotic and endometrial stromal cells from women with endometriosis display reduced capacity for decidualization (Klemmt et al. 2006). In comparison to stromal cells from healthy endometrium, stromal cells of ovarian endometriomas, peritoneal surface lesions and deeply infiltrating lesions have a diminished ability to decidualize (Klemmt et al. 2006).

Recent work has found reduced levels of all-trans retinoic acid (ATRA) in endometriotic lesions (Pierzchalski et al. 2014). This is consistent with our work from our lab, which demonstrated that the retinoic signaling pathway is severely disrupted in endometriosis, leading to an overall decrease in genes involved in retinoid uptake and metabolism (Pavone et al. 2010b, 2011). Specifically, we have shown significant differences in genes involved in retinoid uptake, metabolism and action in endometriosis compared to normal endometrium. We have also demonstrated that paracrine factors, including retinoids, secreted from progesterone-treated endometrial stromal cells act on epithelium to alter steroid hormone response. These factors are absent in endometriotic stromal cells (Cheng et al. 2007). We also have preliminary data showing that the expression of key genes in the retinoic acid signaling pathway are significantly lower in eutopic endometrium of baboons with experimentally induced endometriosis compared to controls during the window of implantation (Pavone et al. 2010a). We hypothesize that decidualization alters the expression of integral genes in the retinoid signaling pathway. We hypothesize that even during decidualization, endometriotic cells would demonstrate a molecular pattern consistent with decreased uptake and metabolism. We believe that these differences may ultimately contribute to the lower implantation rates seen clinically in endometriosis.

**Materials and methods**

**Tissue acquisition**

All tissue used for this study was obtained using an Institutional Review Board–approved protocol. Written consent was obtained from women suffering from benign indications before their surgeries. Eutopic endometrium was obtained from hysterectomies (n=17) and eutopic endometrium (endometriosis) was obtained from cyst walls of ovarian endometriomas (n=15) as has been previously described by our group (Pavone et al. 2011). All the patients were premenopausal and were not on any hormonal therapy for at least 3 months prior to surgery. The phase of the menstrual cycle was determined by preoperative history as well as histological evaluation (all samples were in the follicular phase). The average age of the patients was 38 ± 6 years.

**Isolation and culture of primary stromal cells**

Stromal cells were isolated using the protocol reported by Ryan and coworkers (Ryan et al. 1994) with some slight modifications (Ryan et al. 1994, Noble et al. 1997) to maximize yield. Briefly, the tissues were minced and digested with pronase, deoxyribonuclease, collagenase (Sigma) with constant shaking at 37°C for 40 min, and if required, with hyaluronidase (Sigma) for another 20 additional minutes. Filtration through 70- and 20-µm sieves was used to separate stromal cells from epithelial cells. Stromal cells were then re-suspended in DMEM/F12 1:1 (Life Technology) containing 10% fetal bovine serum (FBS) with 100 µg/mL streptomycin and 100 IU/mL penicillin (Dyson et al. 2014). A humidified atmosphere at 5% CO₂ and 37°C was used to grow the cells. Before the stromal cells were used for any experiments, they were passaged at least once.

**In vitro decidualization**

**In vitro** decidualization (IVD) protocol used was similar as previously reported (Kim et al. 2007). Both human endometrial stromal cells (HESC) and endometriotic stromal cells (OSIS) were grown to 60–80% confluency, and then changed to IVD media consisting of phenol red-free DMEM/F12 media, 2% charcoal-dextran-stripped FBS (cs-FBS) and antibiotics as described above as well as 1 µM medroxyprogesterone acetate (MPA), 35 nM 17β-estradiol (E₂) (both from Sigma) and 0.05 mM 8-bromoadenosine 3',5'-cyclic monophosphate (cAMP, BIOLOG, Life Science Institute, distributed by Axxora, LLC, Farmingdale, NY, USA). The control cells were maintained in cs-FBS media and treated with equal amounts of ethanol as a control (Dyson et al. 2014).

**Small interfering RNA (siRNA) knockdown**

HESC and OSIS were grown to approximately 60–70% confluency. They were then transfected with either a non-targeting negative control siRNA (scCIT) (Dharmacon, Lafayette, CO, USA), siRNA against cellular retinoic acid-binding protein 2 (CRABP2) or fatty acid-binding protein 5 (FABP5) (both from Dharmacon) at a final concentration of 50 nM using Lipofectamine RNAiMAX (Invitrogen). After 12 h of transfection, complete media was added for 24 h. The cells were then subjected to IVD for a period of 4–6 days depending on the siRNAs used. IVD was performed in 2% cs-FBS. The cells were then harvested for RNA and proteins. Media was also collected for ELISA.

**RNA extraction and quantitative real-time RT-PCR**

Total RNA from HESC and OSIS was isolated using RNeasy columns (Qiagen) according to manufacturer’s protocol. One microgram of RNA was used to generate cDNA using q-script cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA). Real-time quantitative PCR was performed using Quant Studio 12K Flex and ABI Power Syber Green gene expression systems (Applied Biosystem). mRNA levels were quantified for prolactin (PRL), FABP5, CRABP2,
retinol-binding protein 4 (RBP4), cytochrome P450, family 26, subfamily A, polypeptide 1 (CYP26A1) and 18S. We have used 18S as the control in many other papers (Pavone et al., 2010b, 2011). Commercially available primers were used for all genes (Qiagen). Comparative threshold (CT) cycles method was used to perform relative quantification of mRNA species. For each sample, the gene CT value was normalized using the formula: ΔCT = CT gene of interest – CT18S (housekeeping gene). The following formula was used to determine relative expression level: ΔΔCT = ΔCT sample – ΔCT. This calibrator was used to plot the gene expression using the 2−ΔΔCT formula. For IVD time course experiments, figures are normalized to mRNA expression on day 0, which we used as a control.

**Preparation of protein**

Whole-cell lysates were prepared for protein quantification. Cells were washed with PBS homogenized using RIPA buffer (1% NP-40, 0.5% sodium deoxycholate, 50 mM Tris, pH 7.6, 0.1% SDS and 150 mM NaCl) supplemented with protease inhibitor cocktail (Sigma Aldrich). The lysates were centrifuged at 14,000 g for 10 min. Equal amounts of protein were resolved on NuPAGE Novex 4–12% Bis–Tris Gels (Life Technologies). Transfer and membrane blocking were performed as described (Dyson et al., 2008). Incubation with primary antibodies was performed at 4°C overnight (cellular retinol-binding protein 1 (CRBP1)) from Santa Cruz Biotechnology; Cytochrome P450; CRABP2, Family 26, Subfamily B, Polypeptide 1 (CYP26B1), from Sigma Aldrich (now Millipore Sigma), FABP5 from Abcam; β-actin from Proteintech Group Inc (IL, USA) after which the membranes were washed and incubated with the appropriate HRP-conjugated secondary antibodies.

**Epithelial cell culture and treatments**

Endometrial epithelial Ishikawa cell lines (a gift from Dr Masato Nishida Kasumigaura National Hospital, Tsuchiura, Ibaraki, Japan) were cultured in monolayers at 37°C, 5% CO2 incubator in DMEM and F12 (1:1) medium with 5% FBS, 1% sodium pyruvate and 1% penicillin–streptomycin and hygromycin antibiotics solution (Life Technologies). After the cell reached about 80% confluency, they were treated with RBP4 (Cayman Chemical) at doses of 0.01–4 µg/mL for 48 h and real-time RT-PCR was done to test for HSD17B2. A time course was also done by treating Ishikawa cells with 1 µg/mL serum RBP4 for 48 h and testing the gene expression levels of HSD17B2 at 1, 12, 24 and 48 h respectively.

**ELISA**

Media was collected from the cell culture plates and protein levels of prolactin (Alpha Diagnostics, San Antonio, TX, USA) and RBP4 (EMD Millipore) from endometrial and endometriotic cells were measured according to manufacturer’s protocol. For IVD time course experiments, we normalized the protein values to the day 0 time point.

![Figure 1](https://example.com/figure1.png)

*Figure 1 In vitro decidualization (IVD) induces prolactin gene expression and secretion in endometrial stromal cells and a much smaller induction in endometriotic stromal cells. Human endometrial stromal cells showed an increase in (A) prolactin (PRL) mRNA levels (shown as fold change), (B) Prolactin mRNA levels (shown as fold change). (C) Prolactin protein concentrations (ng/mL) when compared to endometriotic stromal cell. These experiments represent replications done in 14 patients.*
Statistical analysis

Non-parametric testing (Kruskal–Wallis) was used to assess for statistical significance. A P value <0.05 was considered statistically significant. Error bars represent standard error of the mean.

Results

**IVD treatment increased PRL expression in HESC and to a much lesser extent in OSIS**

As expected, PRL mRNA and protein secretion increased in both HESC and OSIS cells (Fig. 1), although to a far lesser extent in OSIS cells.

**IVD treatment altered expression of genes in the retinoic acid signaling pathway**

Figure 2 shows protein expression of CRABP2, CRBP1 and CYP26B1 when HESC and OSIS were subjected to a 14-day IVD treatment. Interestingly, we observed that in HESC, there was an increase in CRBP1 protein expression while CRABP2 protein expression initially increased and then decreased. In OSIS, expression of both of these proteins was much lower than that in HESC. FABP5 protein expression remained stable in both HESC and OSIS during IVD. CYP26B1 expression is greater in OSIS compared to HESC.

**Knockdown of CRABP2 in HESC cells decreases the ability of cells to decidualize**

We previously showed that CRABP2 is integral in determining cell fate in our model of endometrium and endometriosis (Pavone et al. 2010b). Specifically, we showed that the CRABP2:FABP5 ratio is different in endometrium and endometriosis, with a higher CRABP2:FABP5 ratio in HESC and the reverse in OSIS. Further, we showed that when this ratio was altered, apoptosis and proliferation were affected. We also showed that the expression of CRABP2 is decreased in OSIS compared to HESC. In order to examine if this decreased expression affected decidualization, CRABP2 was knocked down in HESC for 48 h, and cells were then decidualized for 4 days. As shown in Fig. 3B, there was a significant decrease in the prolactin mRNA expression when CRAPB2 was knocked down prior to IVD, indicating a decrease in the ability to decidualize. Results were confirmed by performing an ELISA for protein expression (Fig. 3C). This suggests that CRABP2 expression is integral for proper decidualization to occur and that a decreased expression of this gene prior to decidualization would affect the cell’s ability to properly decidualize.

**Knockdown of FABP5 in OSIS cells increases the ability of cells to decidualize**

We have previously showed that FABP5 is also important in determining cell fate in our model of endometrium and endometriosis. Specifically, we demonstrated that when FABP5 was ablated in endometriosis, apoptotic markers increased and proliferation was decreased. Therefore, we explored what would happen when FABP5 was knocked down in OSIS. We knocked down endogenous FABP5 mRNA in OSIS for a period of 48 h. After the knock down period, the cells were subjected to the IVD treatment for 4 days. As shown in Fig. 4, there was a significant increase in PRL mRNA (Fig. 4B) and protein (Fig. 4C). This suggests that decreasing FABP5 expression in endometriotic cells may increase the ability of the cells to decidualize. However, we do note that the level of prolactin secretion upon FABP5 knockdown is minimal when compared to decidualizing eutopic cells. This, together with the results above, suggests that the CRABP2:FABP5 ratio may affect the cell’s ability to decidualize for both endometrial and endometriotic cells.

Figure 2 Key proteins involved in retinoid trafficking in endometrial and endometriotic stromal cells during *in vitro* decidualization. HESC and OSIS cells show key changes in proteins involved in the retinoic acid signaling pathway during 14-day *in vitro* decidualization treatments. These experiments represent replications done in 10 different patients.
Decidualization induces RBP4 secretion

As shown in Fig. 5, previous work has demonstrated that in normal endometrium, progesterone acts on stromal cells to induce the secretion of paracrine factors that in turn act on neighboring epithelial cells to induce the expression of the enzyme 17β-hydroxysteroid...

Figure 3 CRABP2 knockdown in endometrial stromal cells compromises decidualization. (A) CRABP2 mRNA expression (shown as fold change) is reduced even after 4 days in IVD cocktail. (B) PRL mRNA (shown as fold change) decreases significantly when CRABP2 expression is ablated prior to IVD. (C) These results are confirmed by measuring PRL by ELISA (ng/mL). These experiments represent replications done in 6 different patients.

Figure 4 FABP5 knockdown in endometriotic cells induces decidualization. (A) FABP5 mRNA expression (shown as fold change) is reduced even after 4 days in IVD cocktail. (B) Prolactin mRNA (shown as fold change) increases significantly when FABP5 expression is ablated prior to IVD. (C) These results are confirmed by measuring PRL by ELISA (ng/mL). These experiments represent replications done in 6 different patients.
dehydrogenase type 2 (HSD17B2), which catalyzed the conversion of estradiol to estrone (a much less potent estrogen) (Cheng et al. 2007). In endometriosis, this enzyme is not induced with progesterone treatment because of a defect in paracrine signaling from stromal cells. One of these paracrine factors was thought to be retinoic acid. Retinol-binding protein RBP is another paracrine factor acting as a specific carrier for retinol in the blood, and it has recently been shown that it can act as a signal to other cells (Yang et al. 2005). Interestingly, we found that RBP4 was secreted during the decidualization of HESC cells, and a corresponding increase was not found in OSIS cells (Fig. 5). We then incubated epithelial Ishikawa cells (same cells which had been used by Cheng et al.) with human serum RBP4 for 48h (time point established by Cheng et al.) and found that HSD17B2 expression significantly increased (Fig. 5). We also found a dose-dependent induction (Fig. 5). This suggests that RBP4 may be an important paracrine factor secreted by endometrial stromal cells to induce expression of HSD17B2. RBP4 may be providing retinol that is metabolized into retinoic acid in epithelial cells. In addition, lack of its expression and secretion in endometriotic stromal cells would lead to a lack of HSD17B2 expression and pathologically elevated local estradiol levels. RBP4 treatment also induced CYP26A1 epithelial expression, another RA target gene (Supplementary Fig. 1, see section on supplementary data given at the end of this article).

Discussion

In this paper, we found that the genes in the retinoic acid signaling pathway are altered by decidualization (Fig. 6). Based on our prior work, we chose to focus on the actions of CRABP2 and FABPS for this paper (Pavone et al. 2010b, 2011). We found a consistent pattern of decreased expression of genes involved in retinoid uptake and action, and an increase in the expression of genes involved in retinoic acid elimination in endometriotic stromal cells subjected to decidualization. Taken together, this suggests that even during decidualization, overall retinoid exposure in endometriotic cells is less than that in normal endometrial cells. We also showed that expression of CRABP2 and FABP5 may be integral for proper decidualization to occur. We found that changing this ratio in endometrial cells decreased their ability to decidualize, while altering this ratio in endometriotic cells increased their ability to decidualize, as measured by prolactin expression and secretion. In addition, we found that decidualization induces the expression and secretion of RBP4 in endometrial stromal cells and that RBP4 induces HSD17B2 expression. This suggests that RBP4 may be the paracrine factor secreted...
Endometriosis and decidualization

Decidualization in both eutopic and ectopic endometrium has been described in the setting of endometriosis. Here, we show that a number of proteins in the retinoic acid signaling cascade are altered by decidualization in both endometrial and endometriotic stromal cells (Fig. 2). We have previously shown that endometriotic cells express a molecular pattern consistent with decreased retinoid uptake, metabolism and action (Pavone et al. 2011). Here, we show that this is also true during decidualization. Namely, endometriotic stromal cells overall had a decrease in the expression of CRABP2 and CRBP1 with an increase in the expression of CYP26B1, which allows for the elimination of retinoids from the cell. Pierzchalski and coworkers recently demonstrated defective ATRA biosynthesis in endometriotic lesions related to reduced expression of CRBP1. These studies suggest that defects in this gene resulted in abnormal retinoid biosynthesis and that the ability of endometrial stromal cells to synthesize ATRA correlated well with their degree of decidualization (Zheng et al. 2000, Pierzchalski et al. 2014). Overall our prior studies, as well as these other findings, support the notion that during decidualization the retinoid signaling pathway is disrupted in endometriotic stromal cells. In addition, Sidell and coworkers found RA to be necessary for vascular endometrial growth factor (VEGF) secretion, which, along with endometrial angiogenesis, plays a critical role in successful implantation (Sidell et al. 2010). After ovulation, VEGF production is largely limited to epithelial cells. This is consistent with our findings that RA metabolism is critical for proper decidualization to occur and that RA metabolism in epithelial cells during decidualization is crucial.

There is molecular dysregulation of the proliferative-to-secretory transition in women with endometriosis, suggesting resistance to progesterone action (Klemmt et al. 2006, Aghajanova et al. 2009, Yin et al. 2012). Together with resistance to progesterone action, a deficiency in progesterone receptor (PR) has also been found in endometriotic tissues. It has been suggested that PR deficiency may be responsible for increased proliferation and resistance to apoptosis seen in endometriotic tissues (Bulun et al. 2006). We have previously shown that CRABP2 is regulated by PR and that the resistance to apoptosis may, in part, be explained by decreased expression of CRABP2 in endometriotic cells (Pavone et al. 2010b). We have demonstrated that the ratio of CRABP2:FABP5 is integral for determining cell fate in stromal cells from normal endometrium and endometriotic tissues. We showed that RA may promote differentiation and apoptosis in normal endometrium via CRABP2, while in endometriosis, this pathway is shifted toward FABP5, which mediated anti-apoptotic activity. In addition, we have previously shown that this is independent of nuclear receptor expression, namely RAR and PPAR β/δ (Pavone et al. 2010b). In addition, it has

by stromal cells that stimulates epithelial HSD17B2 expression (Fig. 6).

Endometriosis is associated with pelvic pain and infertility. At least part of infertility is because of a decrease in the ability of an embryo to properly implant. Historically, endometrium has been investigated using an endometrial biopsy during the ‘window of implantation’, where normal histology was thought to be a reassuring finding. However, more and more studies are finding that although histologically normal, the endometrium of women during this window of implantation may be biochemically abnormal and that this may contribute to implantation failure. Lessey and coworkers have demonstrated that even histologically normal endometrium from women with endometriosis lacks the αvβ3 integrin, which is a marker of uterine receptivity (Lessey et al. 1992). Several other genes have been shown to be aberrantly expressed in the endometrium of women with endometriosis, including aromatase, HSD17B2, leukemia inhibitory factor and progesterone receptors (Stewart 1994, Giudice et al. 2002).

Human endometrium undergoes decidualization under the influence of progesterone (Aghajanova et al. 2009). This can be done in vitro, as has been done in our study and many others (Fig. 1) (Aghajanova et al. 2009, Pavone et al. 2010b). Although progesterone is a key hormone in regulating the decidualization process, it has been demonstrated at the molecular level that other pathways as well as other transcription factors are involved. As previously mentioned, impaired

---

**Figure 6** Schematic diagram of endometrial cells during decidualization (in the presence of E2 and P4). Genes in the retinoid pathway are altered during decidualization. During decidualization, CRABP2:FABP5 ratios are integral for proper decidualization to occur. Decidualization also induces the expression and secretion of RBP4 in endometrial stromal cells and that RBP4 induces HSD17B2 expression in nearby epithelial cells. This suggests that RBP4 may be the paracrine factor secreted by stromal cells that stimulates epithelial HSD17B2 expression. Impaired decidualization in endometriotic cells may be, in part, mediated by genes involved in the retinoic acid signaling cascade.
been reported that CRABP2 expression was correlated with the gain of ability to synthesize RA, and that this was important for the process of embryo implantation (Bucco et al. 1996, 1997). Here, we show that CRABP2 expression is key for decidualization to occur properly. By decreasing CRABP2 expression prior to exposing cells to IVD media, prolactin expression and secretion were significantly decreased, suggesting a reduced ability to decidualize (Fig. 3). Interestingly, we have also demonstrated that by decreasing FABP5 expression prior to inducing decidualization in endometriotic cells, we were able to increase the expression and secretion of prolactin, suggesting an induction of decidualization (Fig. 4), although to a much lesser extent than what occurs in HESC.

HSD17B2, which is normally present in endometrial glandular cells but lacking in endometriotic cells, inactivates estradiol. The lack of its presence in endometriotic lesions contributes to increased local estradiol levels. In this paper and others, we have shown that the retinoic acid signaling pathway is integral for proper HSD17B2 expression (Cheng et al. 2007). Here, we suggest that RBP4 may be the paracrine factor that transports retinol to the nearby epithelial cells. This retinol would be metabolized to RA, which would induce the expression of HSD17B2, leading to estradiol inactivation (Figs 5 and 6). We have previously demonstrated that epithelial cells do express genes involved in retinol uptake and metabolism (Mittal et al. 2014). Absence of the expression and secretion of RBP4 from endometriotic cells would contribute to increased local estradiol present in endometriotic cells.

RBP4 is the only specific transport protein for vitamin A, or retinol, in the circulation. It functions to deliver retinol to tissues. Here, we suggest that RBP4 may be the paracrine factor secreted by decidualized endometrial stromal cells that activates HSD17B2 to ultimately decrease local estradiol levels. RBP4 has been shown to act as a paracrine factor in other studies (Yang et al. 2005, Ma et al. 2016). Specifically, it has been suggested that RBP4 may be a signal that contributes to the pathogenesis of type 2 diabetes (Yang et al. 2005). Work by Berry and coworkers also supports the notion that RBP can act as a signaling molecule and is able to deliver retinol to cells even in the absence of STRA6 (Berry et al. 2013). Additionally, study found that fenretinide, a synthetic retinide, normalizes serum RBP4 levels and improves insulin resistance and glucose tolerance in mice with obesity induced by a high-fat diet (Yang et al. 2005). We have recently shown that treatment of endometriotic lesions with fenretinide induces apoptosis. In vivo treatments were found to decrease lesion volume, and we hypothesized that an increase in retinol uptake may be the underlying mechanism of action (Pavone et al. 2010a). Taken together with the results by a study by Yangand coworkers, this suggests that increasing retinol uptake may also increase retinol secretion and thus increase RBP4 secretion.

The major strength of this study is that all experiments were conducted using human tissues. One limitation is that we were unable to conduct the studies in eutopic endometrium from women with endometriosis. In our facility, most women with endometriosis undergoing uterine surgeries are also on medical management for endometriosis and pelvic pain, thus not ideal candidates for these types of studies. However, as mentioned in the introduction, we do have in vivo data using a baboon model, which supports our findings (Pavone et al. 2010a). In addition, we were unable to measure actual retinoids in this study. We hope to be able to do this in the future.

In summary, we have demonstrated that gene expression in the retinoic acid signaling pathway is altered by decidualization. We suggest that impaired decidualization in endometriotic cells may be, in part, mediated by genes in the retinoic acid signaling cascade. We showed that expression of CRABP2 and FABP5 may regulate the cells’ ability to properly decidualize. We have also demonstrated that RBP4 may be an important paracrine factor secreted by stromal cells to regulate epithelial expression of HSD17B2. We believe that these molecular differences occurring in endometriotic stromal cells during decidualization could help explain the clinical decrease in implantation seen in women suffering from this disorder.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/REP-16-0592.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This work was partially funded by ASRM Career Development Award, Friends of Prentice (to M E P); K12HD050121 (to M E P; PI SEB); R37HD038691 (to S E B).

References
Aghajanova L, Hamilton A, Kwintkiewicz J, Vo K & Giudice L 2009 Steroidogenic enzyme and key decidualization marker dysregulation in endometrial stromal cells from women with versus without


Pavone ME, Pearson E, Cheng YH, Fazleabas A & Bulun S 2010a Retinoic acid action is deficient in endometrium in a baboon endometriosis model. Reproductive Sciences 17 157A.


Received 2 November 2016
First decision 23 November 2017
Revised manuscript received 31 May 2017
Accepted 6 June 2017